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THE FLAGELLATE FAUNA OF THE CŒCUM OF THE STRIPED GROUND SQUIRREL, *CITELLUS TRIDECEMLINEATUS*, WITH SPECIAL REFERENCE TO *CHILOMASTIX MAGNA* SP. NOV.

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The intestinal protozoan fauna of the rat, mouse, guinea pig and rabbit has been investigated quite extensively. Not so much has been done on field rodents as on those which lend themselves to laboratory use. In the autumn of 1925 and spring of 1926 the writer made a study of the protozoa harbored in the alimentary tract of the striped ground squirrel, *Citellus tridecemlineatus*. This rodent was found to be quite as rich a field for investigation of protozoan parasites as the aforementioned animals.

The protozoa so far found to be residents of the cœcum of the striped ground squirrel comprise one species of amœba, *Endamœba citelli;* one species of *Chilomastix;* two species of *Trichomonas*, one of them probably new; one species of *Tetratrichomastix*, subgenus of *Eutrichomastix;* one species of *Hexamitus;* and a flagellate which is either a species of *Hexamitus* or *Urophagus*. In the small intestine a *Giardia* was found. *Endamœba citelli*, and its pathogenic parasite, *Sphærita endamœbæ*, were described by the writer (1926) in a previous paper. The *Giardia* was sent for identification to Dr. R. W. Hegner, who found it to be a new species.* The *Chilomastix*, *Trichomonas*, *Tetratrichomastix*, and *Hexamitus* will be reported upon in this paper in the order named. The preparations of the other flagellate were not entirely suitable

*Since this paper went to press a paper by Dr. R. W. Hegner has appeared in which this *Giardia* is described. See Hegner, R. W. 1926. *Giardia beckeri* n. sp from the ground squirrel and *Endamœba dipodomysi* n sp. from the kangaroo rat Jour. Paras. 12: 203–206.

for critical study, and the specimens were very scarce. Consequently a description of this species (*Hexamitus* or *Urophagus*) must be delayed until more suitable material is obtained. Twenty ground squirrels in all were examined for protozoa. All save one harbored infinite numbers of protozoa in the cœcum. This one had only a light infection.

The technique consisted of a careful microscopic examination of thin emulsions of the cœcal contents in normal salt solution. Permanent prepared slides were made by Heidenhain's iron-hæmatoxylin staining after fixation in Schaudinn's solution.

Chilomastix magna sp. nov. This protozoan was found in all twenty ground squirrels. The shape is essentially like that of *Chilomastix mesnili* from man, pyriform with three flagella at the broadly rounded anterior end. There is often a tuft of short bristly cuticular projections in the region of the flagella (Fig. 1). It may be present in either living or stained material, and is not a fixation artefact. Fixed and stained individuals measured from 10 to 21.6 micra in length and from 6 to 11 micra in width, the average width being about 9 micra. The length of *Chilomastix mesnili*, as given by Kofoid and Swezy (1920) is from 9.6 to 15 micra in stained preparations. The writer measured a number of *Chilomastix mesnili* on slides which he had prepared, and found a range of from 7 to 11 micra. When one considers that twenty out of thirty specimens of *Chilomastix magna* measured from 15 to 21.6 micra, it will be seen that this flagellate is almost twice as long as the one found in the intestine of man.

A study of the cytology of this protozoan promised to be particularly interesting because of the discrepancies between the accounts of *Chilomastix mesnili* by Kofoid and Swezy (1920) and Dobell and O'Connor (1921). The latter authors are particularly outspoken in flatly denying the existence of certain cell structures which the former claim to have seen. Without desiring to become in any way involved in the controversies of these writers, the writer must state that his observations agree more nearly with those of Kofoid and Swezy, although his interpretations may differ in certain respects. The morphology of the free form, cyst, and dividing individuals will be described.

The nucleus of the free form lies in the extreme anterior end of the body. Measurements indicate a diameter of from 3 to 4.5 micra. Anterior to the nucleus lie a number of deeply staining granules, concerning the affinities of which there is so much controversy. Kofoid and Swezy's account of the arrangement of these granules based largely upon their observations of the cyst is somewhat as follows. There lies upon the nuclear membrane a centrosome, which is connected by the "nuclear rhizoplast" with a "primary blepharoplast." This blepharoplast is joined to the "secondary blepharoplast" by means of the "transverse rhizoplast.". Finally, another thin thread-like line connects the secondary and tertiary blepharoplast. Directed somewhat posteriorly from the secondary and tertiary rhizoplast are two deeply staining fibrils, the one (parastyle) supporting the left lip of the cytostome, the other (parabasal body) supporting the right lip. Two of the three anterior flagella take their origin in the primary blepharoplast, one from the secondary. From the tertiary rhizoplast there arises, in addition to the "parabasal body," the cytostomal flagellum and a thread enclosing the mouth, the "peristomal fiber." The cytostomal flagellum vibrates along the edge of an undulating membrane. Dobell and O'Connor deny the existence of any integrated fibrillar system (neuromotor system), or connections between the granules at the anterior end, claiming in addition that there are six granules instead of four. Furthermore, in regard to the peristomal fiber they state, "We believe there is no such fibre, and that the mouth is not situated in this position."

Despite long and painstaking study the writer was not able to make out all the details of the granule complex with the positiveness of Kofoid and Swezy. That there is an integrated fibrillar system connecting the granules, however, the writer has no doubt. The rhizoplast connecting the blepharoplasts designated by Kofoid as secondary and tertiary is especially prominent in most specimens because of its thickness and deeply-staining qualities (Fig. 12). A fine rhizoplast running from a granule on the nuclear membrane was occasionally recognized. The best evidence for the fibrillar system, however, comes from the dividing stages, which will be described below. The writer was not able to make out the exact affinities of the flagella, because of the impossibility of resolving the granules in the granule complex. Fig. 12 represents one of the best observations which the writer was able to make.

A structure was noted which Dobell and O'Connor neither mention nor figure, but which resembled very much Kofoid and Swezy's figures of the peristomal fibril enclosing a more deeply-shaded granular area, the cytostome. In *Chilomastix magna*, this structure takes its origin in the blepharoplast complex, and passes posteriorly just dorsal to the cytostome and right supporting fibril (Kofoid's "parabasal body"), if we consider the cytostome to be on the ventral side. It usually extends far beyond the posterior curvature of this fibril into the posterior region of the body (Fig. 1). It appears to be surrounded by a fibril. This structure, however, has three dimensions, and is from one third to one half as deep as broad, as proved by polar views of a number of flagellates standing on end. So what appears to be a fibril, is in reality a membrane marking the boundary between this structure and the remaining cytoplasm. Rarely this structure appears to adhere to the right supporting fibril (Fig. 2). In others, no connection between the two is evident (Fig. 1). There is no connection between this body and the cytostomal flagellum, which lies between the right and left supporting fibrils (Fig. 1, 2, 12).

What is the function of this organelle? Is it an oral structure associated with the mechanism for the intake of food? It is not visible in the unstained living specimens, so this point could not be determined. Is it a reserve food supply? Failure to stain it either with iodine or *intra vitam* with Janus green gives no clue. For the present the writer prefers to call it a "parabasal body," because of its non-committal significance of anything as to function, although it does not stain with Janus green, which Shipley (1916) found would color the parabasal body of *Trypanosoma lewisi* and Becker (1923) found would color the parabasal bodies of *Crithidia* and *Herpetomonas*, suggesting their mitochondrial nature. Besides this structure, no other organelle resembling a peristomal fiber was seen. Might it not be that this body is much shorter in *Chilomastix mesnili*, and that the sharpness of its limiting membrane led Kofoid and Swezy to call it a peristomal fiber?

The cyst is typically lemon-shaped (Fig. 6). The size is markedly constant, every mature cyst measuring almost exactly 10 by 7.5 micra except one, which measured 12 by 8 micra. By way of comparison the writer measured twelve cysts of *Chilomastix mesnili*. These measured from 6 by 5 micra up to 8 by 7 micra. This indicates that the cyst of *Chilomastix magna* is somewhat larger than that of *Chilomastix mesnili*, but there is not the difference in size that there is in the free stages. The cyst contains a nucleus, the cytostomal structures, and the "parabasal body."

There is nowhere in the literature anything like a complete account of the cell division of *Chilomastix*. For this reason it was thought to be quite worth while to spend a large amount of time searching for dividing specimens, although they were extremely rare. As a result a fairly complete account of the process can now be given, although not all points have been cleared up.

What the writer conceives to be the resting nucleus is represented in Figs. 1, 7, and 12. There may be one blob of chromatin on the inner anterior surface of the nuclear membrane, or there may be one blob here and another in the posterior portion of the nucleus (Figs. 1, 12), or there may be a large number of smaller granules lying on the nuclear membrane (Fig. 7). There is a cloud of chromatin granules scattered thruout the entire nucleus. Very often at this time a small deeply staining spot, an endobasal body, or intranuclear kinetic element is visible (Figs. 7, 12).

A little later the granules become more concentrated and form a large endosome (Figs. 2, 9, 10). Careful study of this endosome shows that it is sometimes a concentration of granules (Fig. 2) and sometimes apparently a "chromatin knot" (Figs. 9, 10). Often a deeply-staining intradesmose is visible at the points where it passes from the endosome to the blobs of chromatin at one or both poles of the nucleus.

The endosomal chromatin becomes resolved into chromosomes, which appear to be dumbbell-shaped. It was not possible to count them exactly in any case, but the number is near ten. Strangely enough, the intradesmose could not be observed at this stage. Perhaps it lay too close to the nuclear membrane, for which there is some evidence to be given later.

The chromosomes arrange themselves in an equatorial plate, and a spindle forms. Fig. 11 is an exact drawing of the spindle at the time it first comes into evidence. The poles of the spindle are the blobs of chromatin at the poles of the nucleus in which the intradesmose had previously terminated. There is some evidence that these blobs of chromatin are not the centrioles, but that the centriole lies within them; *e.g.*, Fig. 2 shows what appear to be two centrioles lying against the chromatin blobs.

As mitosis progresses the spindle elongates considerably, the nucleus elongating simultaneously and the nuclear membrane commencing to dissolve. The polar blobs of chromatin disappear, leaving the tiny centrioles at the poles of the spindle (Fig. 3). The chromosomes of the dividing equatorial plate of the early anaphase become massed together so that it is not possible to count them, although in some cases they may appear as a mass of discrete granules.

The oral apparatus of the parent cell (right and left supporting fibrils, blepharoplasts, etc.) has degenerated. The centrioles divide, leaving connecting rhizoplasts between each centriole and its daughter blepharoplast. The daughter blepharoplast divides at least once (perhaps more), and from the products of the division the left and right supporting fibrils of the mouth grow out. The intradesmose is in evidence along the nuclear membrane, connecting the centrosomes at the poles of the spindle.

The intrazonal spindle fibers disappear in the late anaphase, and the two halves of the equatorial plate are disconnected from one another (Fig. 4). The remains of the spindle attaches the chromatin mass to the centrioles, while a fine rhizoplast connects the centriole with the blepharoplast complex. Fig. 4 appears inconsistent with Fig. 3 in regard to the points of attachment of the intradesmose which traverses the cell. It is extremely difficult to resolve the granules exactly, and the writer has put the intradesmose in each case exactly where it appeared to be. At this stage the "parabasal body" has appeared, and lies in its permanent position, just dorsal to the right supporting fiber.

The cell constricts in the middle (Fig. 5). The two daughter cells then reconstruct themselves, each with a complete new set of cell organelles. During division no food particles can be seen in the cell protoplasm.

Trichomonas muris (Hartmann) var. *citelli*. This flagellate was encountered in only three out of twenty ground squirrels. It is a common inhabitant of the cœcum of albino, house, and wild mice. The form from the ground squirrel, however, so closely resembles in its important features Wenrich's (1924) description of *T. muris* that it is considered to be at least a variety of this species. Furthermore, the chromosome count agrees with the number which Wenrich found in *T. muris*. The size of *T. muris* on prepared slides, according to Wenrich, ranges from 8 to 22 micra, with an average of 12.9 micra. The average of the body length of 100 individuals from the ground squirrel, not including the protruding axostyle, was 17 micra, somewhat larger than Wenrich's measurements.

The reader is referred to Wenrich's excellent account for the detailed description of this species. A rhizoplast connecting the nucleus and blepharoplast was seen by the writer in some cases. Andrews (1925) also saw it in *T. termopsidis*. One other rather unimportant respect in which the form from the ground squirrel differs from Wenrich's description is in the arrangement of the "inner row of chromatic granules." In the form studied by the writer the granules in this area constitute a field rather than a linear series (Fig. 13). In some specimens a small granular body was noted near the anterior end, although Schaudinn's fixative was used (Fig. 13). This was interpreted to be the parabasal body, as described by Wenrich. What the writer saw was evidently a residue left after the mitochondrial substance had been dissolved out. This parabasal body, however, does not stain *intra vitam* with Janus green.

Large numbers of dividing individuals were found. The writer has nothing to add to Wenrich's account. Several good chromosomes counts were made at the anaphase (Fig. 14) and prophase (Fig. 15). The number was six, in agreement with Wenrich. There seemed to be in most cases a fairly definite size range for the prophase chromosomes from one large chromosome down to a very small one (Fig. 15). Multiple mitosis or somatella formation of the nature of that observed and described by Kofoid (1915) was not seen in this material.

Trichomonas sp.? This small flagellate was present usually in large numbers in all ground squirrels examined. The body is elliptical, with a pointed posterior end, due to the protruding axostyle (Fig. 19). Length is from 6.5 to 10 micra without the axostyle which protrudes from 2 to 3 micra beyond the body. The nucleus lies at the anterior end. In the resting stage there is a round central chromatic endosome, an achromatic nuclear membrane, with a space between the endosome and membrane which is clear except for a sprinkling of fine granules. The endosome is prominently visible even in living unstained specimens. A large deeply staining basal body lies anterior to the nucleus. From it arise an axostyle which curves about the nucleus, and passes to the posterior end of the body; three anterior flagella; and a fourth flagellum which is directed toward the posterior. It lies in close contact with the body for about half the length of the cell, and the remaining length trails behind.

Typically, members of the genus *Trichomonas* possess an undulating membrane with a chromatic basal rod; *e.g.*, *Trichomonas muris*. This flagellate possesses no true undulating membrane, since the posterior flagellum which is the homologue of the marginal flagellum lies close to the body. It vibrates only slightly in the anterior region, while the free posterior end is quite active. Stained preparations show no chromatic basal rod. Whether these differences are sufficient to create a new genus is doubtful. Stained preparations often show a large open cytostome (Fig. 17). In living preparations it is usually closed.

Very few dividing forms were found, and hence no chromosome counts were made. Sometimes this flagellate is parasitized by a Sphærita (Fig. 19).

Tetratrichomastix citelli sp. nov. This flagellate (Figs. 16, 18) was present in nineteen of the twenty ground squirrels. The body is irregularly pyriform, but approximately bilaterally symmetrical, due to a slight indentation just behind the anterior tip of the body from which the flagella leave the body. The axostyle protrudes from the posterior tip of the body. The body length, exclusive of axostyle, ranges from 7 to 13 micra. The axostyle often extends 2 to 4 micra beyond the body. The flagella are five in number, four anterior ones which beat backwards in unison, with lashing strokes, and a *schleppgeisel*, which does not come so far forward as the others in its lashings. The flagella arise from a blepharoplast situated on the anterior surface of the nucleus. It is almost impossible to count the flagella in the living cell, because of their peculiar movements. A large number of favorable observations in the stained specimens makes it fairly certain that the number is five.

The nucleus is round, and possesses a tiny endobasal body embedded in a mass of granules (Fig. 16). Sometimes only a larger deeply staining endosome is seen (Fig. 18). About the nucleus is sometimes seen a cloud of granules (Fig. 16).

The axostyle passes from the anterior end to the posterior tip. It stains lightly. A number of favorable observations show that it originates in the basal granule.

A cytostome is often indistinctly seen at the anterior end.

Hexamitus pulcher sp. nov. This *Hexamitus*, found in eighteen of the twenty ground squirrels, is characterized by the presence in the living cell of a large number of slightly refringent spherules which stain *intra vitam* green with Janus green at first, then later are reduced to the red. These granules dissolve out for the most part in Schaudinn's fixative, although the cytoplasm of the stained cells is markedly granular (Figs. 20, 21). It was because of the wealth of mitochondria and the beauty of the vital stain with Janus green that the specific name *pulcher* was given. The body is irregularly oval, bilaterally symmetrical, and somewhat plastic. The size does not vary much from eight to ten micra in length by six or seven micra in width.

The flagella are eight in number, the anterior pair projecting almost at right angles to the body and vibrating actively in the living cell. Two other pairs are inclined to wrap themselves about the body giving it the deceptive appearance of possessing several undulating membranes. The posterior pair leaves the body at the posterior tip. Between them the posterior tips of the axostyles protrude.

Stained slides show the cell structures more plainly (Figs. 20, 21). In the anterior region on each side of the median line is an elongated, deeply staining, nucleus. Between the nuclei lie a pair of blepharoplasts from which the flagella arise, altho sometimes there seem to be two pairs. A pair of axostyles in the axis of the body terminate at about the location of the blepharoplasts. In the anterior tip of the cell is a dark spot connected to each axostyle by a fine fiber.

Summary.

The intestines of twenty ground squirrels (*Citellus tridecemlineatus*) were examined for protozoa. The following species of protozoa were found to be present: *Giardia* sp.?, *Endamœba citelli*, *Chilomastix magna* sp. nov., *Trichomonas muris* var. *citelli*, *Trichomonas* sp.?, *Tetratrichomastix* (*Eutrichomastix*) *citelli* sp. nov., and *Hexamitus pulcher* sp. nov. The first was found in the small intestine, all the others in the cœcum. In this paper all the above species except the first two are described, particular attention having been given to the morphology of the free *Chilomastix magna* and to its division phases.

So far as is known, all these protozoa are commensals. Whether or not they assist in the digestion of cellulose, which is said to take place very largely in the cœca of certain animals, is unknown.

Bibliography.

Andrews, J. M.

'25 Morphology and Mitosis in *Trichomonas termopsidis*, an Intestinal Flagellate of the Termite, *Termopsis*. Biol. Bull., **59**: 69–85.

Becker, Elery R.

'23 Observations on the Life Cycle of *Crithidia gerridis* Patton in the Water-strider, *Gerris remigis* Say. Jour. Paras., **9**: 141–152.

'23 Observations on the Life History of *Herpetomonas muscæ-domesticæ* in North American Muscoid Flies. Jour. Paras., **9**: 199–213.

Dobell, C., and O'Connor, F. W.

'21 The Intestinal Protozoa of Man. London.

Kofoid, C. A., and Swezy, O.

'15 Mitosis and Multiple Fission in Trichomonad Flagellates. Proc. Am. Acad. Arts and Sc., **51**: 289–378.

'20 On the Morphology and Mitosis of *Chilomastix mesnili* (Wenyon). Univ. Calif. Pub. Zoöl., **20**: 117–144.

Shipley, P. G.

'16 Vital Staining in *Trypanosoma lewisi*. Anat. Rec., **10**: 439–445.

Wenrich, D. H.

'21 The Structure and Division of *Trichomonas muris* (Hartmann). Jour. Morph., **36**: 119–147.

EXPLANATION OF PLATE.

× ca. 1950.

Fig. 1. *Chilomastix magna*, free form, showing a tuft of cortical projections in flagellar region, and dorsal view of the cytostomal structures. Large granular body dorsal to cytostomal organelles is the "parabasal body."

Fig. 2. *Chilomastix magna*, free form, dorsal view with "parabasal body" apparently following right supporting fiber of cytostome.

Figs. 3, 4, 5. *Chilomastix magna*, free form, in division.

Fig. 6. *Chilomastix magna*, cyst.

Figs. 7 to 11. (Not according to scale.) Various nuclear appearances found in *Chilomastix magna*.

Fig. 12. (Not according to scale.) Dorsal view of nucleus, integrated fibrillar system and cytostomal structures of *Chilomastix magna*.

Fig. 13. *Trichomonas muris* var. *citelli*.

Fig. 14. *Trichomonas muris* var. *citelli*, dividing, nucleus in anaphase.

Fig. 15. *Trichomonas muris*, prophase nucleus of *Trichomonas muris* showing six chromosomes.

Figs. 16, 18. *Tetratrichomastix citelli*, free forms.

Figs. 17, 19. *Trichomonas* sp.? Individual in Fig. 19 infected by a *Sphærita*.

Fig. 20. *Hexamitus pulcher*, side view.

Fig. 21. *Hexamitus pulcher*, front view.